The Implications Of Using Synthetic Biology As A Method Of Water Treatment

University of York iGEM

Human Practices report 2014

Contents

Project summary

Introduction

This report contains our full research into the implications of using synthetic biology as a method of water treatment. We have fully looked into the effects that cadmium and foreign bacteria would have on humans and ecosystems in order to conclude if it is worth using genetically modified bacteria in such applications. Research has also been done to investigate public perceptions of such methods of remediation.

Brief description of our project

Our project is entitled EcoCADMUS (*E. coli* Cadmium DecontaMination Univeral System) it targets metal and sulfate contaminated wastewater, generated both from industry and mineral processing, which is a worldwide problem. Cadmium is one of the most commonly encountered toxic heavy metals in contaminated water and is known to cause severe damage to aquatic biodiversity and to humans. Furthermore, the removal of contaminants from wastewater is mainly based on mechanical/chemical processes that present drawbacks such as inefficiency, high energy costs and high technological cost. The University of York iGEM team has developed a new approach to remove cadmium and sulfates from wastewater using genetically engineered *Escherichia coli*. Our circuit is activated by a cadmium inducible promoter and is divided into two interconnected mechanisms:

(i) increasing sulphate uptake and its targeting into cysteine production by engineering the cysteine biosynthesis pathway;

(ii) increasing cadmium uptake and targeting of the free-cysteine into metal binding proteins (phytochelatins) for metal chelation/stabilization inside and outside cell .

Safety impacts

Impact of using bacteria in water on humans

The organism we have used for our project is *Escherichia coli K-12*, one of the bacterial model organisms widely used in microbiology and molecular genetics research. Unlike the wild type strains, which cause food poisoning, this particular strain is no longer pathogenic. The risk assessment of the derivatives of *E. coli K-12*[1] shows that the strain is incapable of colonising the human gastrointestinal tract, because it lacks cell wall components that are necessary for attachment to the intestinal walls.

That is not to say that *E. coli K-12* is completely harmless. The fast evolution and mutation rates of E. coli cause the mean fitness of the bacteria to increase without limits, as proven by Lenski's long-term evolution experiment[2] Lenski has been examining the genetic changes in 12 initially identical populations of *E. coli* for 26 years (spanning over 60,000 bacterial generations). All of the populations have increased their adaptability, and one of the populations has even evolved to use citric acid as a carbon source in aerobic conditions. This suggests that even though *E. coli K-12* is non-pathogenic in normal conditions, the bacteria could evolve and develop new characteristics, which could allow them to successfully inhabit the human gastrointestinal tract. A recent study by Koli et al[3] states that *E. coli K-12* can become pathogenic and invade mammalian cells by altering its cellular transcription pattern, with relatively few changes in its genome.

Overall, even though *E. coli K-12* is considered a strain with no or low risk to individuals, caution must be exercised before and during the release of the bacteria into water supplies.

Impact of cadmium in water on humans

Out of all possible heavy metal pollutants, Cadmium (Cd) is one of the most concerning to public health.

Firstly, it works in a strong dose-dependent manner [4], and requires much lower thresholds of exposure than when compared with other heavy metal pollutants (Ie: lead, mercury, hexavalent chromium, etc.). Secondly, the half-life of Cd in the human body is extremely long, approximatively 20 yrs. As a result it accumulates chronically and acutely into the human body primarily in the liver and kidney. This net accumulation tends to be irreversible[4] though some elimination can occur through urea (0.005 to 0.01 per cent of the total body concentration levels).

Concentration of Cd in these organs is due to the high levels of metallothionein (MT), a low molecular weight (500 - 14,000 Da), cysteine-rich protein family located in the Golgi apparatus membrane.

As MT is a small protein it quickly is extracted from plasma via glomerular filtration before being taken up by the proximal tubular cells. Due to this glomerular-filtration pathway, Cd accumulates selectively in the renal cortex via concentration in these proximal tubular cells.
It has been determined that the kidney is the primary storage organ of Cd concentration. Thus, it is the first organ to demonstrate signs of toxicity.

The effects of Cd concentration are not felt until a certain threshold level whose estimate ranges from 150-200 ppm (µg/g wet weight of renal cortex), in both human and experimental animal subjects. [5][6][7]

Early development of Cd-induced critical damage is determined from the presence of microproteins in an abnormal renal condition called tubular proteinuria. These microproteins include β2 -microglobulin, retinol-binding protein and alpha1- macroglobulin. When the rate of excretion of these micro proteins becomes too high, early stage Cd nephropathy (damage to or disease of the kidney) becomes irreversible. Increases in the rate of excretion of these proteins result in a decreased glomerular filtration rate. This GFR can continue to deteriorate with age.

Cd nephropathy subjects excrete other solutes; albumin, amino acids, enzymes (i.e. N-acetyl-β-D glucosaminidase), glucose, tubular antigens, phosphate and calcium in greater amounts than the tubular proteinuria micro proteins

The excretion of phosphate and calcium leads to a disruption in the bone mineralization. Issues with kidney stone and bone fracture formations have been observed.[8]

Effects of Cd contamination on bone density became apparent with the observation of Itai-Itai disease in Japan[9]. Severe osteomalacia (bone softening) was recorded along with multiple bone fractures and renal dysfunction symptoms. Other symptoms include back pain, pain in the extremities, walking difficulties, and pain when pressure is applied to the bones.

Bone effects result from Cd nephropathy, due to an altered vitamin D metabolism and the urinary waste of calcium and phosphate, especially at high levels of Cd exposure.

Itai-itai renal abnormalities can include glycosuria (the excretion of glucose in urea).

Cd is classified as a known carcinogenic source. When Cd is administered by various routes, it can produce cancer at multiple sites, including lungs. [10]

As Cd is not highly genotoxic and does not cause direct genetic damage, epigenetic mechanisms and/or indirect genotoxic mechanisms such as blockage of apoptosis, alterations in cell signalling or inhibition of DNA repair may be involved. [10]

As of yet, no adverse effects of Cd exposure on the nervous or reproductive system has been found. [5]

Comparisons of overall impact on humans

The use of Genetically Modified Organisms (GMOs) in wastewater treatment as proposed by iGEM York has one obvious advantage; the removal of Cadmium. However, there are disadvantages that arise from the use of GMOs that must be considered also.

<u>Disadvantages</u>

Due to the extremely toxic nature of high Cadmium levels in the water any disadvantages to the GMO apparatus will be caused by the GMO itself. It is no secret that GMOs have faced considerable criticism from the general public who don't trust the practices of Synthetic Biology to remain enclosed and sustainable in the global ecosystem. According to data from the survey carried out by iGEM York, the biggest populations against the use of GMO are people with careers or other significant connections to science. This is surprising as many anti-GMO arguments are

of poor scientific foundation [11]. The lack of trust in GMO usage may hinder the widespread deployment of this technology in wastewater processing.

A similar ethical issue related to the biological safety of water is the fluoridation of water to prevent tooth cavities and pits a conflict between the common good and individual rights [12]. Water fluoridation is common practice in the United States, United Kingdom, Ireland, Canada and Australia but has been halted in many countries after substantial protest from the population. This precedent case highlights the public's wariness and suspicion to accept modification to life's most basic requirement; clean, drinkable water.

One of the many arguments from anti-GMO supporters is the fear that there may be unforeseen problems arising from the use of GMOs in the global ecosystem. There exists a stringent set of rules that all GMOs must be tested against before they can be used while contained in the laboratory [13]. Of course, given the complexity of cell biology there is always a possibility for unexpected results to occur. For these occasions, many GMOs are designed with a 'kill-switch', which may be a dependence on a certain chemical not found in nature or by another mechanism. The basic premise of the kill-switch is that unexpected escape of the GMO can be controlled by ensuring that the organism cannot survive in the outside world.

<u>Advantages</u>

Cadmium is a seriously toxic poison which can be contained and the incidence of Cadmium poisoning reduced. Although they are currently methods for wastewater treatment to remove Cadmium, our method allows retrieval of the Cadmium and allows the recycling and resale of the sequestered Cadmium. This method is also easily adaptable to other heavy metals via the extensive library of genes available to Synthetic Biologists.

Impact of foreign bacteria on ecosystems

Bacteria are already used in wastewater treatment, making our project simultaneously easier and more difficult.

<u>Bacteria use</u>

"Activated sludge"[14] is a combination of bacteria (95%) and, protozoa and other microbes (5%), which remove small organic molecules from wastewater. The treatment environment directly affects the microorganisms within the sludge and as such, the treatment plants are designed specifically to contain the organisms. Aerobic bacteria are moved through an aeration tanks where they thrive and multiply before settling at the bottom of the clarifier tank. By the time they settle at the base of the clarifier tank, most of the nutrients and oxygen in the water has been used up[12]. Introduction of a new bacterial species will require the supply of greater quantities of nutrients and oxygen to the system[14]. This alteration to the environment will not be as big a challenge of initial introduction of bacteria as all of the necessary tools for altering the environment are already in place. However, it many require careful consideration to ensure the environment maximises productivity of the organisms. The current problems in environment which can occur for activated sludge are[15]:

- Poor primary clarification
- Hydraulic overload
- Nitrification
- Nutrient shortage
- Organic overload
- Cold weather
- Organic under load

Technology is already in place to deal with these problems however; potentially expensive tests may need to be undergone in order to find the most efficient environment for the new organisms[16]

Another problem with having organisms already present in the wastewater environment is that the *E. coli* will be in competition. We do not know the implications of this but if we were to introduce our bacteria into an industrial environment, we would ideally model this initially. It would also be useful to model the speed of uptake of cadmium, and therefore clarification of the water. This would allow us to work out how rapidly the water would ideally move through the system.

GMO usage

The fact that the *E. coli* we would be introducing would be genetically engineered would introduce a further layer of complexity. Because bacteria will already be present in the treatment plant, there is a risk of transformation of the inserted genes into other species. In order to prevent spread of the genes, we will be introducing a kill switch into the bacteria. This prevents escape from the environment. However, to control the inserted genes, we would also need to ensure these could not be taken up by other organisms in the environment. For this, we have considered containing the bacteria in a semi-permeable membrane through which the DNA would be unable to pass.

Impact of cadmium on ecosystems

Aquatic Ecotoxicity

Studies have shown that Cd is toxic to a variety of organism groups.

Even at low concentrations Cd toxicity extends to all life, including plants, fish, birds, mammals (humans), and microorganisms [17][178[19] Processes of bio- accumulation (when an animal accumulates Cd from the environment into its body) and bio-magnification (concentration of Cd increases from one link in the food chain to another link in the food chain) have been observed. These processes are of increasing concern as one climbs the food chain to the top predator levels, (humans, polar bears, etc.) where the effects of bio-magnification are amplified due to concentrated level accumulation of the pollutant.

Many of these studies have focused on determining Cd contamination effect in aquatic organisms. Across a whole range of different organism groups, the effects of Cd contamination range from accumulation to affecting biochemistry, physiology, feeding behaviour and mortality amongst other effects.

Below is a description of the specific effects Cd has on the specified aquatic organism group.

Fish: present accumulation of Cd levels, biochemical

changes, affected development, changes in feeding behaviour, affected genetics and growth, histology, hormones, immunological responses, injuries, intoxication, affected morphology, physiological changes and impacts on reproduction.

The average acute toxicity for fish is moderately toxic, while the acute toxicity range is slight to very high toxicity.

Crustaceans: display Cd accumulation, behavioural, biochemical, developmental, enzymatic, genetic, growth, histological, and mortality effects. The average acute toxicity for crustaceans is moderately toxic, while the acute toxicity range is slight to very high toxicity.

Marine Bentic Organisms: show mortality effects. The average acute toxicity for marine benthic organisms is highly toxic, while the acute toxicity range is moderate to high toxicity.

Phytoplankton: exhibit accumulation, behavioural, biochemical, cellular, ecosystem processes, enzymatic, growth, mortality, physiology, population and reproduction effects. The average acute toxicity for phytoplankton is not acutely toxic, while the acute toxicity range ranges from not acutely toxic to moderate toxicity.

Zooplancton: present accumulation, behavioural, biochemical, developmental, enzymatic, feeding behaviour, genetic, growth, intoxication, and mortality effects. The average acute toxicity for zooplancton is highly toxic, while the acute toxicity range is moderate to very high toxicity.

Molluscs: present accumulation, behavioral, biochemical, developmental, enzymatic, growth, histological, morphological, mortality, physiological, and reproductive effects. The average acute toxicity for molluscs is moderately toxic, while the acute toxicity range extends from slight to very high toxicity.

Echinoderms: demonstrate accumulation, behavioral, biochemical, developmental, enzymatic, genetic, growth, and reproductive effects.

Annelids: show accumulation, biochemical, genetic, growth, mortality, physiological and reproductive impacts. The average acute toxicity for amphibians is moderately toxic, while the acute toxicity range is slight to very high toxicity

Nematodes/Flatworms: exhibit effects of accumulation, histological, and mortality impacts. The average acute toxicity for amphibians is slightly toxic, while the acute toxicity range goes from not acutely toxic to moderate toxicity.

Amphibians: display accumulation, biochemical and mortality effects. The average acute toxicity for amphibians is very highly toxic, while the acute toxicity range is very highly toxic

Insects: demonstrate accumulation, behavioral, biochemical, developmental, feeding behavior, growth, mortality, physiological, population, and reproductive effects. The average acute toxicity for insects is slightly toxic, while the acute toxicity range is slight to moderate toxicity

Aquatic Plants: show accumulation, behavioral, biochemical, developmental, growth, physiological, population, and reproductive changes.

From these studies it can be seen that even at low doses, cadmium has a wide implication in disturbing ecosystems at the biological level.

Previously mentioned processes of bio-accumulation are particularly concerning, especially in top level food web predators (ie: humans). Since elimination of Cd from tissues is negligible it becomes obvious that the severity of the effects Cadmium induces will continue to increase. When mentioning the phytoplankton example, ever increasing levels of Cadmium could have a greater impact on ecosystem processes in unknown ways.

Designing a system to deal with Cadmium uptake from water would be beneficial in terms of improving Cadmium pollution levels and ultimately would have knock on effects on the global food web and ecosystems. Food security from fish stocks and human health would also see positive gains.

Comparisons of overall impact on ecosystems

One of the primary aims of our project is to create a cleaner environment: one that is better for the organisms living there. It is undeniable that cadmium has harmful impacts on our environment. As shown above, the toxic heavy metal has a whole range of adverse effects on organisms. However, we have also discussed the potential issues with the introduction of a foreign, genetically engineered organism into the environment. When it comes to using new technologies in the environment, the risks and advantages need to be weighed against each other.
Pros and cons of cadmium in the environment.

Though the presence of cadmium in the environment may have no known advantages, there may be good reason not to uptake it. Cadmium only occurs at very low levels in the environment usually. Ambient air cadmium concentrations have generally been estimated to range from 0.1 to 5 ng/m³ in rural areas, from 2 to 15 ng/m³ in urban areas, and from 15 to 150 ng/m³ in industrialised areas[20]. Cadmium is also a natural, usually minor constituent of ground water. At these levels, cadmium does not pose a serious risk to the environment or to human health[21]. Studies have shown that cadmium is taken up by plants, and therefore entire removal from the environment may have unforeseen effects on the ecosystem.

The cases in which a technology such as ours would become invaluable would be situations where cadmium levels were significantly above average. We are looking specifically at cases of cadmium-containing waste spillages. Spillages of cadmium are a genuine risk, with serious occurrences as recently as 2012, when seven chemical company officials were detained over contamination of Longjiang River, China[22]. Avoiding polluted water supplies would not be enough to mitigate disasters as there is evidence that cadmium is capable of moving into the food chain and causing serious health risks such as the sinisterly named Itai-Itai disease[9]. In Japan during the nineteen hundreds, a law suit was brought against a mining and smelting company for cadmium pollution of the environment which contaminated crop plants and resulted in cases of osteomalacia in those consuming the plant. This case illustrates that the heavy metal would not simply be present as an inorganic compound. In fact it would make its way into the food chain having inevitably devastating consequences for the ecosystem as well as nearby people. Cadmium also does not break down in the environment. Particles can be wind-borne and may travel long distances before settling in a new environment. Some forms are also water soluble and can contaminate groundwater[23].

Pros and cons of our technology
The benefits of our technology are clear. There are currently no highly efficient mechanisms for the rapid removal of cadmium from the environment. The advantages of using bacteria are several-fold. Firstly, the systems for implementation in wastewater treatment are already in place. The technology would be self-replicating and therefore complex machinery would not constantly need to be used. The bacteria could be maintained at a constant level and theoretically encouraged into rapid proliferation if a large quantity was needed e.g. for treatment of a waste spillage. The bacteria also chelate the ions of valuable cadmium allowing it to be harvested for use. The metal has several uses in industry including production of batteries and pigments.

However, there are disadvantages to the use of genetic engineering. Perhaps foremost is the controversy surrounding the issue of GMOs. The use of genetic engineering can inevitably be fraught and some businesses will not use anything which involves such methods. This in itself is a problem but signifies others which underlie it. There are risks to genetic engineering, as stated above, which would need to be considered. Insertion of new constructs would, as stated above, result in an added layer of precautions necessary where the technology is used. These extra precautions, such as the use of semi-permeable membranes, might result in added costs to our technology.

People's opinions on uses of bacteria in the environment

In order to identify people's opinions on the uses of bacteria in the environment we used multiple surveys. These surveys allowed us to also identify how people's views differed dependant on their age, gender, nationality, scientific literacy and religious and political views. Our survey was spread through a range of methods in order to obtain as much of a range in views as possible, this included over Facebook, Twitter, through GMO/anti-GMO websites and through other sharing websites. Overall we had 179 responses to our surveys.

1. Environmental Protection

91% of responders considered environmental protection important or extremely important (Figure 1). For this question, significant differences between groups were only observed between genders, with 78% of females saying that environmental protection is extremely important, compared to only 40% of males.

2. Opinion on Environmental Remediation Projects and Initiatives

We asked our responders whether they would support or discourage environmental remediation projects and initiatives. Significant differences were observed between age groups, genders and Western and Eastern Europeans.

95% of responders under the age of 24 said that they would support or strongly support environmental remediation projects, compared to only 73% of those over 24 (Figure 2)

At 92%, females are also more likely than males to support or strongly support environmental protection initiatives, who would only do so 80% of the time (Figure 3).

As opposed to 98% of Western European responders, only 75% of those from Eastern Europe would support projects aiming to protect the environment (Figure 4).

3. Opinion on Environmental Remediation Projects that Rely on GMOs

Although 85% of our responders said that they would support, or strongly support environmental remediation projects and initiatives, the number dropped to 60% when responders were told that these projects would use genetically modified organisms. Compared to the previous question, where there were significant differences between groups based on age, gender and nationality, in this case the only significant differences are based on religious affiliation (Figure 5) and scientific literacy (Figure 6).

For the purposes of this report, we have divided our responders into two categories: religious and non-religious. However, we plan on carrying out more in depth analysis of our data in the near future, when we will further divide the “religious” category based on the specific affiliation indicated by our responders.

As far as scientific literacy is concerned, we asked our responders to select the level of scientific literacy they possess from the following:

1 - little or no contact with science and no understanding of the scientific method
2 - occasional contact with science(eg. media) and a basic understanding of the scientific method
3 - contact with science through secondary education(eg. high school science classes) or equivalent level and a moderate understanding of the scientific method
4 - contact with science through higher education(eg. university science degree) or equivalent level, ability to read and understand articles in scientific journals and a good understanding of the scientific method
5 - an understanding of science equivalent to having a career in a scientific field, ability to read and understand articles in scientific journals, a very good understanding and experience of applying the scientific method.

Although there is no significant difference between levels 2 and 3, responders who have identified with level 4 scientific literacy were significantly more likely to support environmental projects that relied on GMOs and over 80% of those who identified with level 5 scientific literacy said they would support such initiatives. None of our responders have selected level 1 scientific literacy.

Our responders were asked to what extent they had researched GMOs before taking the survey. Our findings show that, as expected, the responders who had done extensive research into the topic were more likely to “Strongly support” or “Strongly discourage” environmental projects that relied on GMOs. Responders who had done little to no research into GMOs were less likely to take a stance on this issue, with 40-50% of them saying that they would neither support nor discourage these projects (Figure 7).

4. Opinion on the Impact of Various Factors on One's Stance towards GMOs

Responders were asked to rate on a scale from 1(lowest) to 5(highest) the impact that they believe certain factors have had on their attitude towards GMOs. Our findings show that up to 70% of our responders believe that their opinion was shaped by scientific consensus as well as independent research. At the other end of the spectrum are religious and political affiliation, with less than 10% of responders saying that these factors have had an impact on their attitude towards GMOs (Figure 8).

5. Additional Comments and Opinions

Our responders were given the opportunity to comment on our survey and on the topic of GMOs. A significant number have expressed their disappointment in the way GMOs are portrayed in the media and in how the concept of GMOs is understood by the public:

“We urgently need more scientifically accurate info throughout the media! Sensationalist fear mongering is a poison and the antidote is lacking.”

“GMO subject is poorly understood by general public and superficially treated by media”

“Having talked to non scientific friends I have found that the vast number are unaware that plants and other organisms have genes. As a consequence they assume that we are putting

genes into foreign bodies and "making them humanoids"

“Generally considered to much more of an unknown terror in England than in America (where I presume you are based?). Viewed by the general public, by spin in the media, as unnatural and hazardous but I think it surely it has to be the future for crop development in a world where population growth is far outstripping food production.”

“Depending on what continent your survey participants are from, some of the answers may have entirely opposite meanings: in the U.S. GMOs are largely accepted as useful and safe (and are therefore legal) while in the E.U. there is a lot of misinformed public opposition to them (and also some legislation aiming to limit or prohibit their use).”

Some of the comments have also expressed the view that the problem with GMOs is not the organism itself, but the way it is used by corporations:

“I don't mind GMOs designed to thrive in harsh environment or have a stable fertile crop. But I do have a problem with copywriting them, designing them to be drowned in pesticides or making the crops sterile as to require the re-purchasing of seed stock ever year. So really it's the business/ politics not the science that bothers me.”

“I think a major factor is the gap between the problems of uses of the technology and whether it has inherent issues - the exploitation of GMOs by major companies is a real problem but has led to excessive fear of the technology.”

How we would deal with public opinions

In a survey we conducted we discovered that 76% (111 out of 147 people asked) of people asked were either comfortable or very comfortable with bacteria that has been genetically modified being released into the environment in a controlled manner. As a result we concluded that we should attempt to find a manner in which we can use our bacteria in a controlled way in the environment.

After consulting our supervisors we concluded that the best way to do this would be by using a hollow fiber bioreactor (HFB), this would allow us to contain or bacterium in a device which we would be able to allow water to flow through therefore allowing our bacteria to take up and cadmium or sulfate ions present in the water. If our bacteria were to escape from the bioreactor it would be disposed of later on in the water treatment process.

A HFB is a device that would allow us to use our bacteria in a controlled manner in the environment as the bacteria is kept within a plastic cartridge, through which tubes approximately 200 microns in diameter are run. These tubes have a semi-permeable membrane around them which permits the movement of small molecules such as ions to pass though so that the cells contained are able to use them[24] (figure 9). Typically these HFBs are used for containing mammalian cells in order to identify any protein product changes if different substrates are used, however we spoke to John Cadwell from Hollow Fiber Systems who informed us that using them for bacteria would be possible if we consider certain limitations including the amount of oxygen that can be supplied to the cells[25]. Therefore we would be able to pump cadmium ion contaminated water through the tubes in the bioreactor which can then pass though the membrane in order to be taken up by our bacteria.

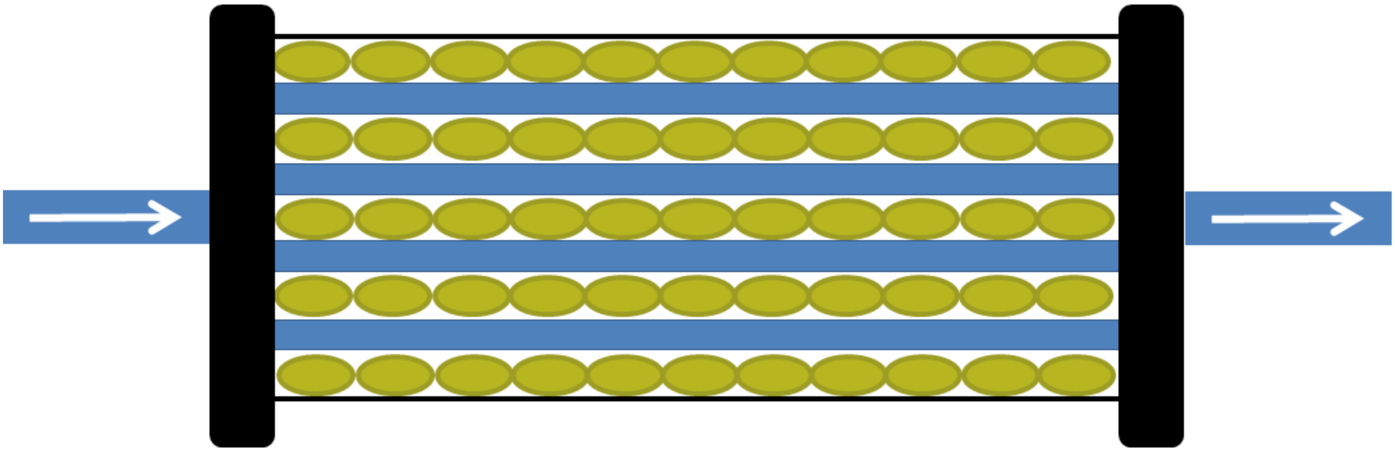

Figure 9. Lateral cross section of a hollow fiber bioreactor containing out bacteria (green) with cadmium contaminated water flowing through it (blue)

Disposal of escaped bacteria

Our hollow fiber bioreactors would be able to be used in the water treatment process if they are used as desired, this would then enable them to remove cadmium from the water before it has a change to affect the drinking water supplies.

When water is treated it first has coagulants added to it which are then mixed into the water, as a result the heavy particles in the water will stick together and will settle at the bottom of the tank.[3] The water that is being treated is then filtered through layers of fine granulated materials so the water can be further purified.[26] We would then place our hollow fiber bioreactor as the next stage of water treatment as if any bacteria were to escape the would then be destroyed when disinfectant is added to the water to destroy any bacteria left.[26] However by this point of the water treatment process all sulfur would have been removed from our waste water,

therefore it would have to be added again and the water then be retreated, which obviously poses a large issue. Furthermore this would only be useful in areas where water is treated before it is used.
As a result of this another method which we could use to stop bacteria entering the waterways is by introducing a kill switch. This is a switch that can be developed into bacteria so that when there is a lack of a certain product the bacteria will be destroyed[5]. This however is further an issue due to the introduction of genetic materials from the destroyed bacteria being free in the environment where other organisms may be able to take it up.

Conclusions

This report provided us with the ability to generally evaluate the safety and sustainability aspects of our project. As a consequence of this we were able to think further into how we could adapt the project further for the future and for industrial purposes.

As far as safety is concerned it is absolutely essential to remove as much cadmium from the water ways as physically possible because of the adverse affects it can have on both humans and ecosystems.

Concerning the uses of foreign bacteria for this application it appears that there should not be too many issues concerning humans. That said however foreign bacteria can have severe adverse affects on ecosystems for example by the formation of 'activated sludge' (page 6). Also there is a further issue of the uses of GM bacteria in the environment. These are both relating to safety and sustainability of our project. As we discovered from out surveys, people are less likely to accept the uses of GMOs in bioremediation that bacteria that has not at all been genetically modified and if the public disapprove of their uses one could argue that it renders the project itself unsustainable in the future.

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Appendix 1: Figures

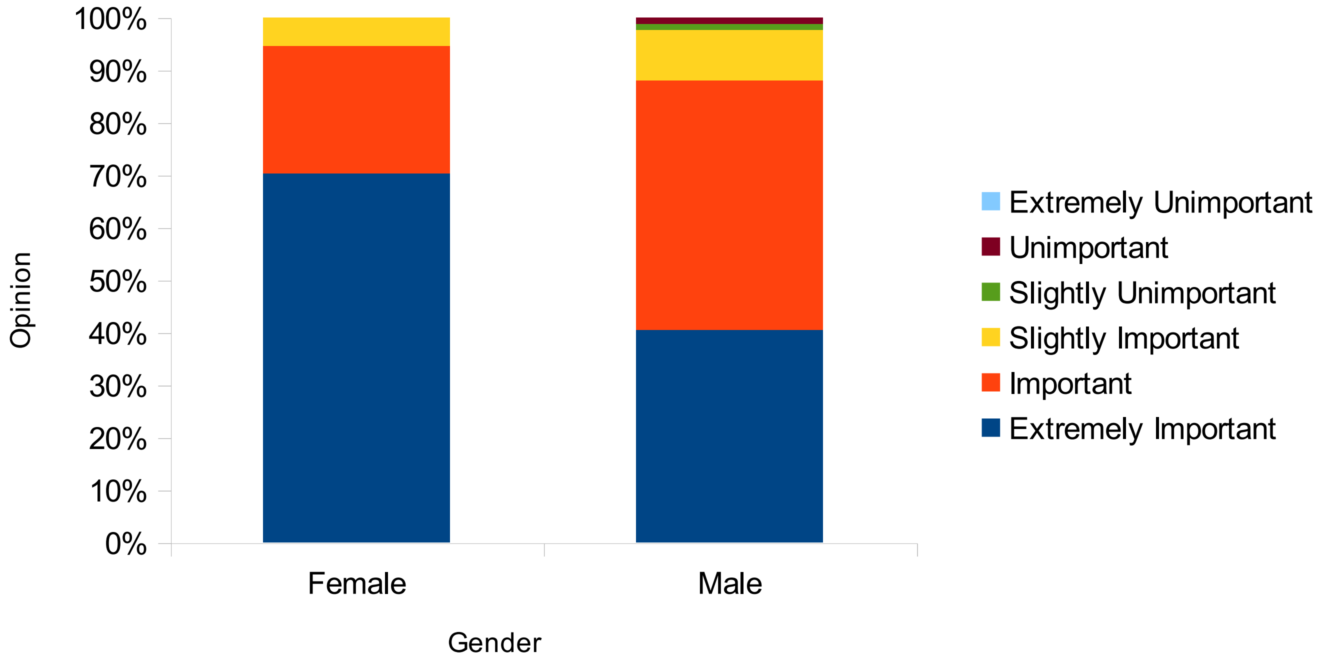

Figure 1. Responses the the question 'In your opinion, protecting the environment is:'

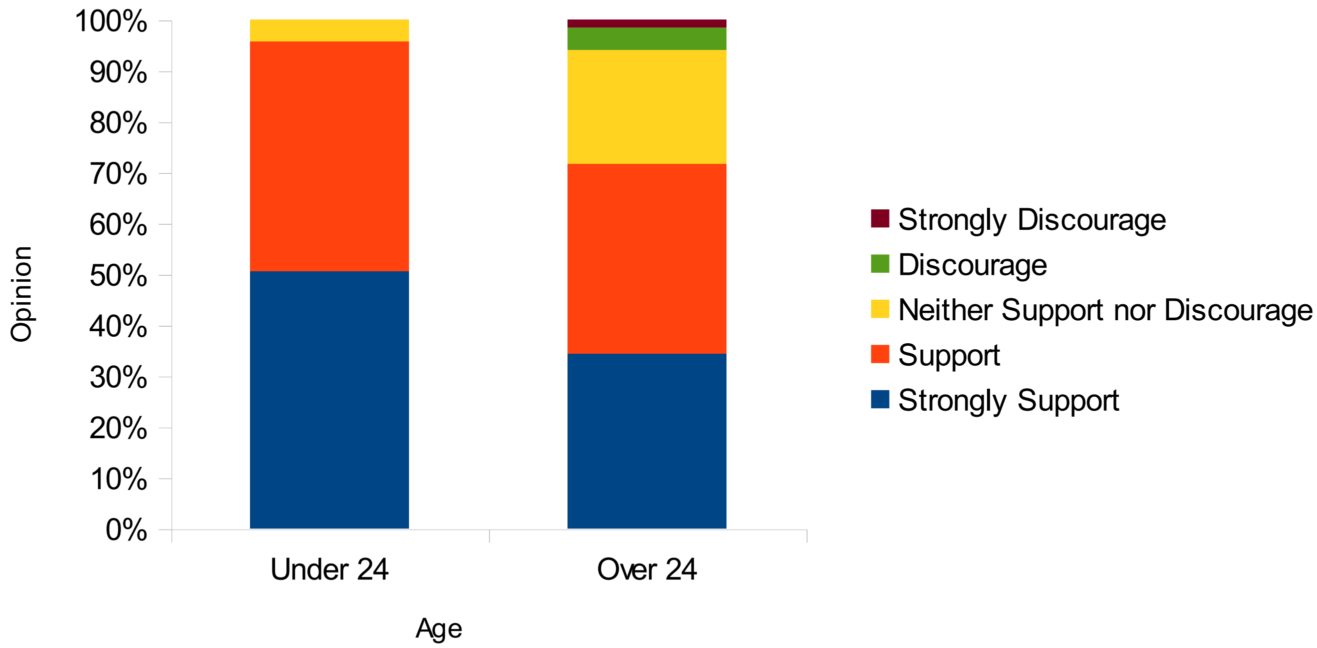

Figure 2. Responses to the question "Would you support or discourage environmental remediation prodjects and initiatives?" Based on age.

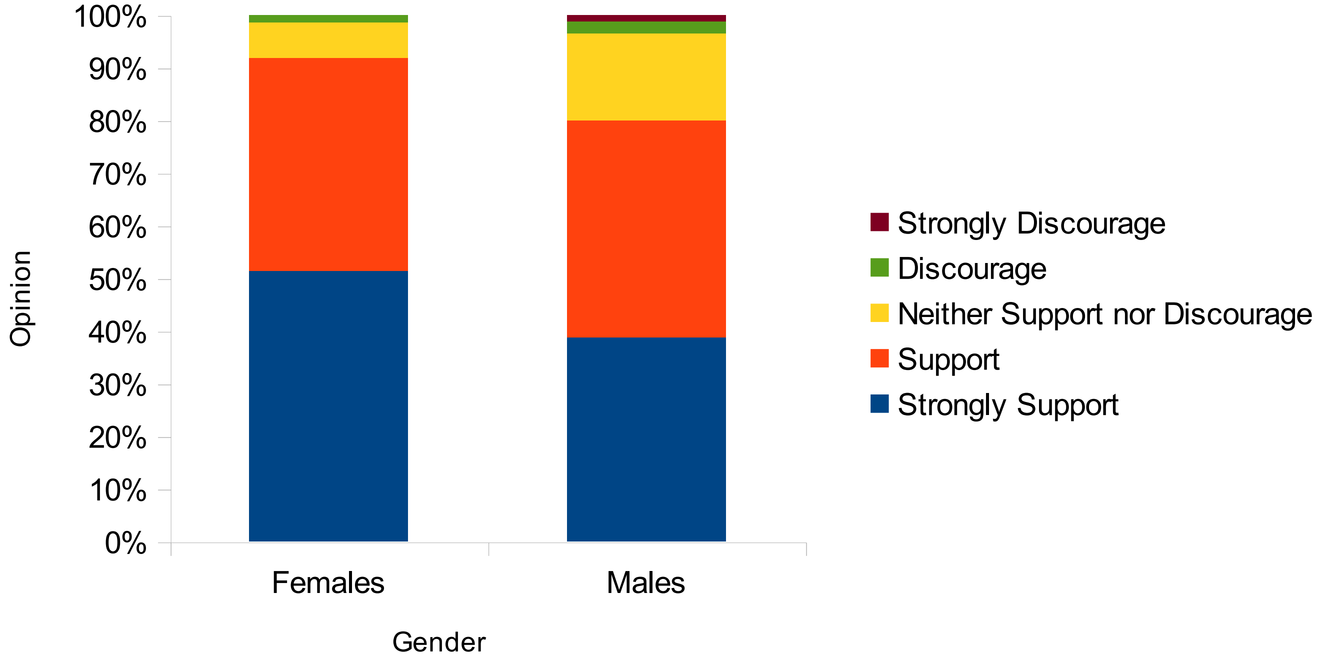

Figure 3. Responses to the question "Would you support or discourage environmental remediation projects and initiatives?" Based on gender.

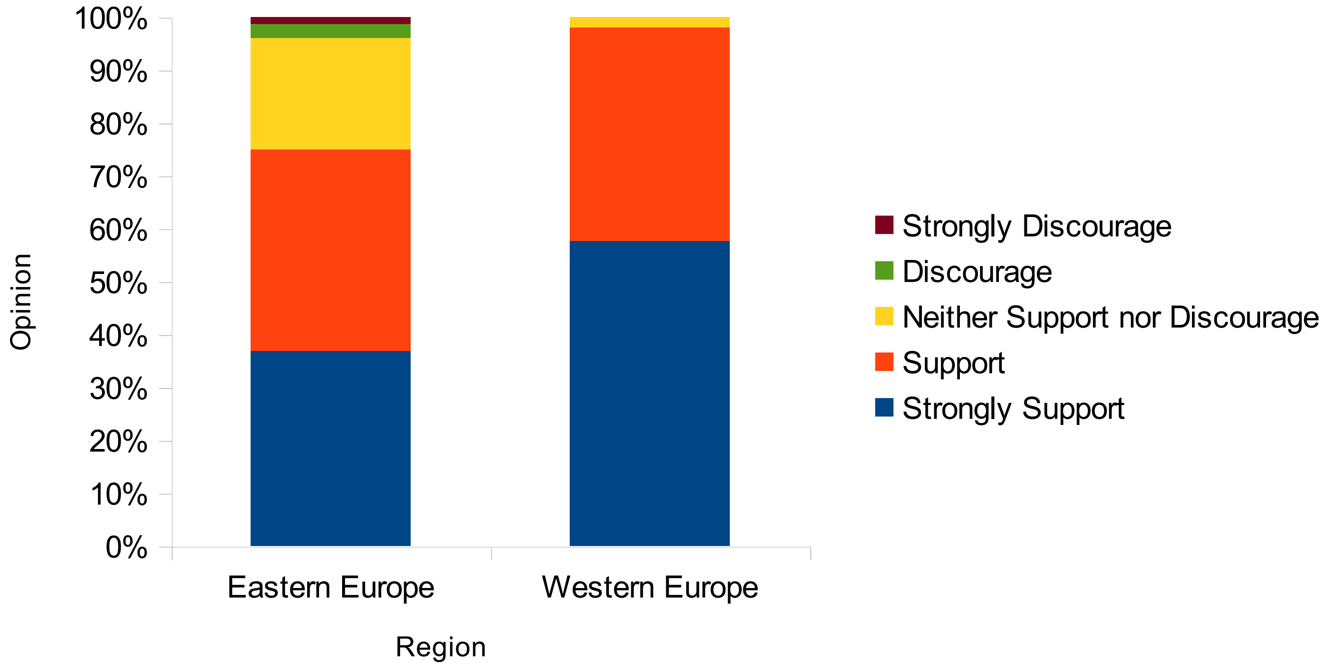

Figure 4. Responses to the question "Would you support or discourage environmental remediation projects and initiatives?" Based on region of origin.

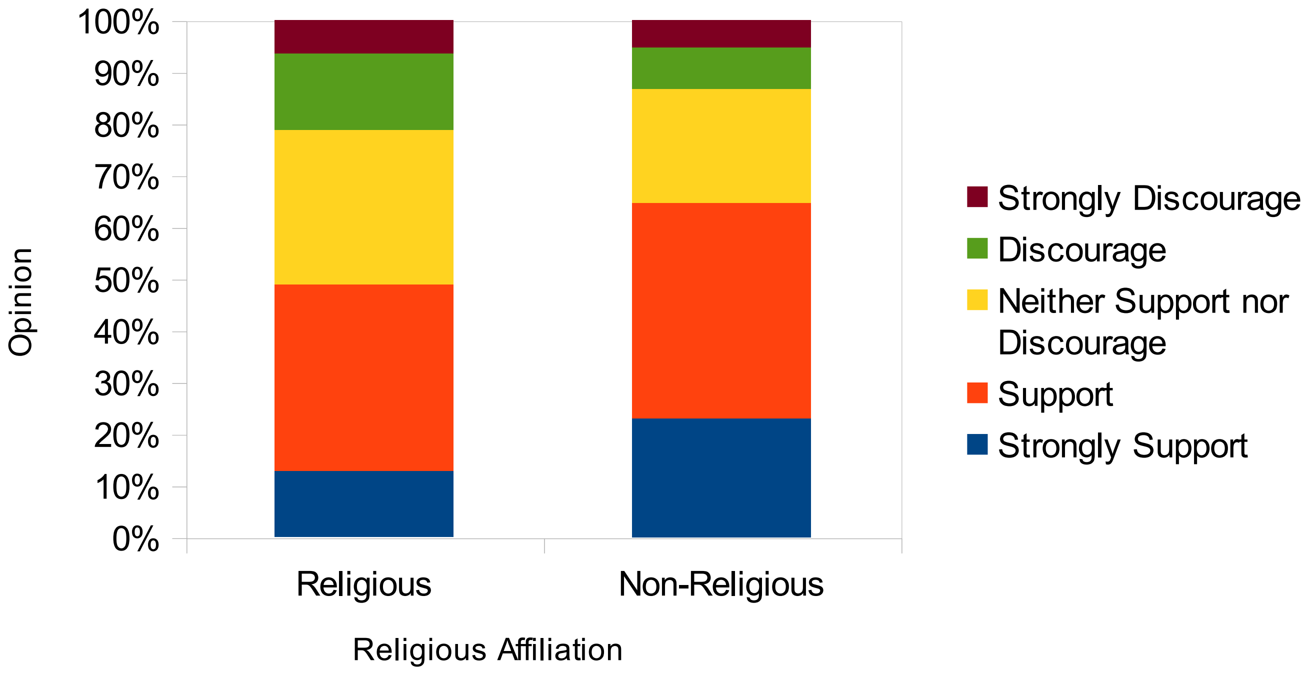

Figure 5. Responses to the question "Would you support or discourage environmental remediation projects and initiatives that rely on the use of GMOs?" (Opinion based on religious affiliation)

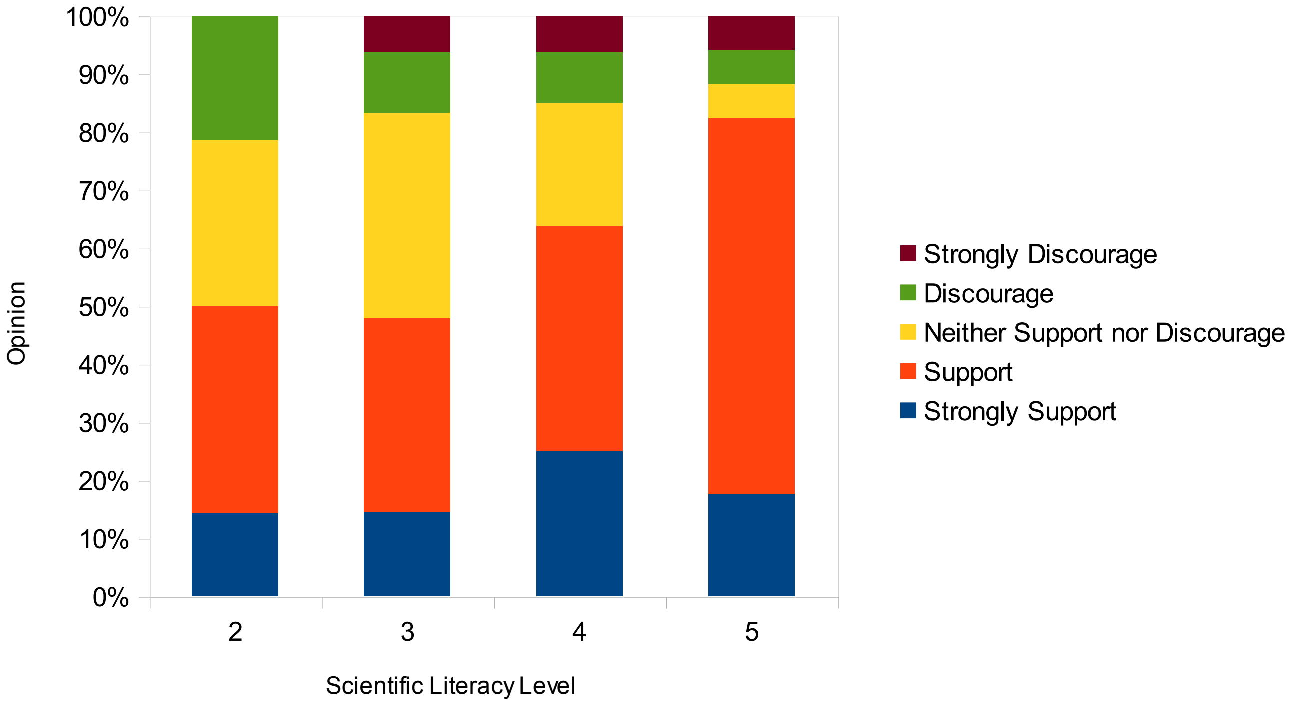

Figure 6. Responses to the question "Would you support or discourage environmental remediation projects and initiatives that rely on the use of GMOs?" (Opinion based on scientific literacy level)

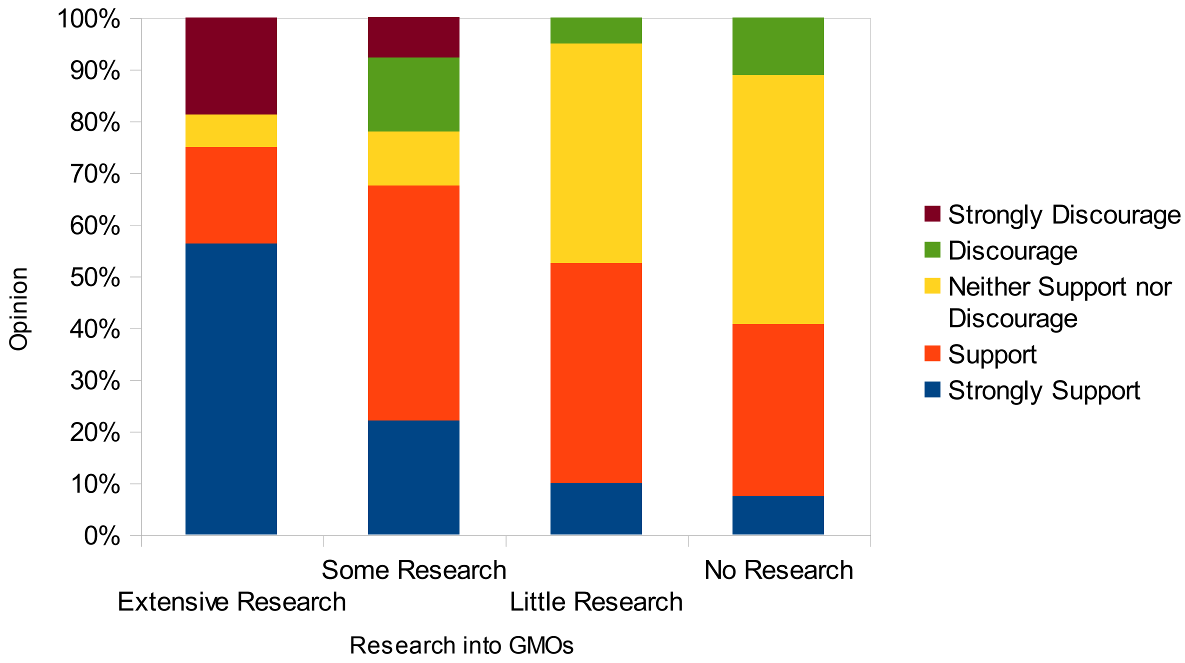

Figure 7. Responses to the question “Would you support or discourage environmental remediation projects and initiatives that rely on the use of GMOs?” (Opinion based on previous research)

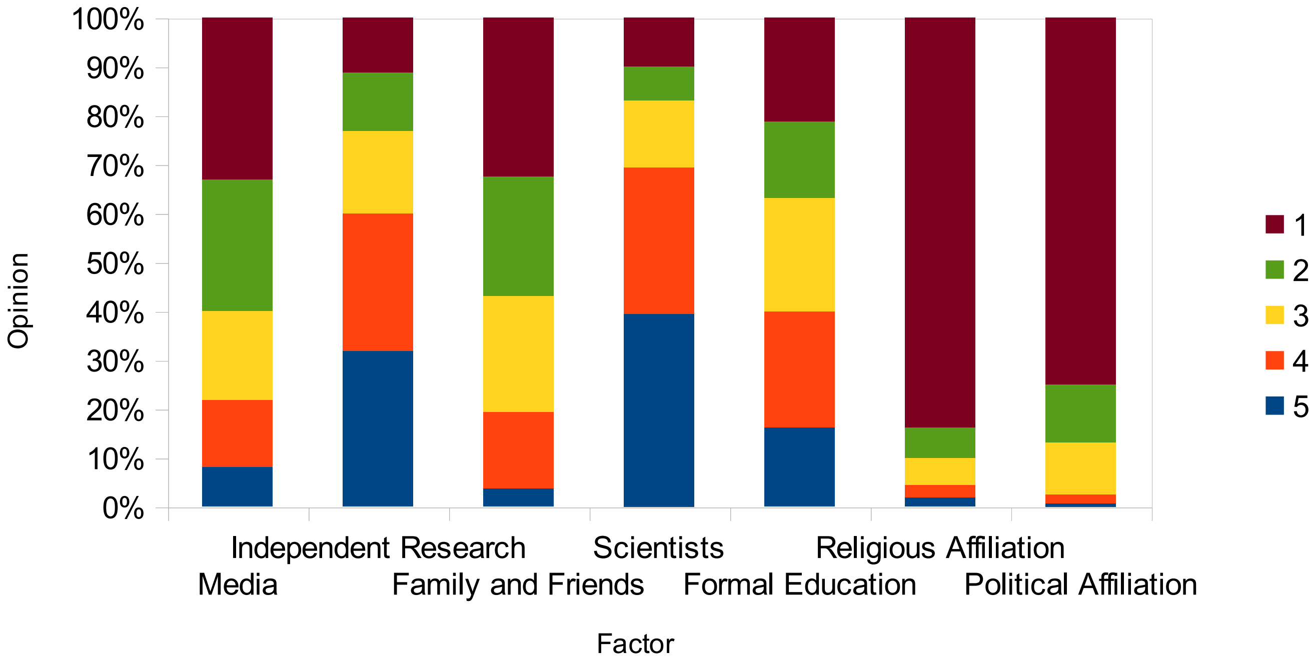

Figure 8. Responses to the question “On a scale from 1(lowest) to 5(highest), how would you rate the impact that each of the below has had on your attitude towards GMOs?”

Appendix 1: Sponsors

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